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ON THE COXO-DORSALIS METATHORACIS MUSCLE IN BEETLES AND ITS POSSIBLE FUNCTION

K. R. MENON AND N. K. JOSHI

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IN beetles as in some other orders of insects the coxa is incorporated in the metathorax, thus enabling the *tergo-coxal* muscles to function as indirect flight muscles. Chadwick (1953) states that this incorporation of the coxa in the body wall, which deprived it of its primitive freedom of movement has enabled the reversion of the original insertion and attachment of these muscles, thus enabling them to function as flight muscles.

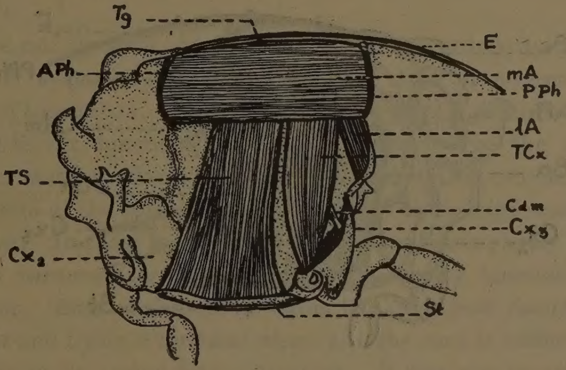

Fig. 1—Median lateral dissection of the Metathorax of *Oryctes rhinoceros*, showing the indirect flight muscles.

Aph—anterior phragma;
Cdm—coxo-dorsalis metathoracis muscle;
Cx_2 — coxa of the mesothoracic leg;
Cx_3 — coxa of the metathoracic leg;
E—Elytra;
lA—lateral oblique muscle;
mA—median longitudinal muscle:
PPh—posterior phragma;
St—Sternum;
Tg—tergum;
TS—tergo-sternal muscle;
TCx—tergo-coxal muscle.

The truth of this statement is borne out by the fact that the best fliers are those in which the coxa is immovably fixed and even incorporated in the body wall. But in the large majority of beetles we find that even though the ventral rim of the coxa is fused with the sternum and the coxal cavity is open and continuous with the cavity of the metathorax, the dorsal rim is free and the whole coxa can move in an antero-posterior direction like one half of a hinge on the other which is fixed. In such forms the ability for long range sustained flight is absent and after a short time they drop down obviously exhausted. Their direct and indirect flight muscles appear to be well-developed as in the good fliers, the striking deficiency being the incomplete fixation of the coxa. It is obvious that if the *tergo-coxal* muscles are to function efficiently as indirect flight muscles, the coxa should either be fixed immovably or it should be held immovable at least during flight.

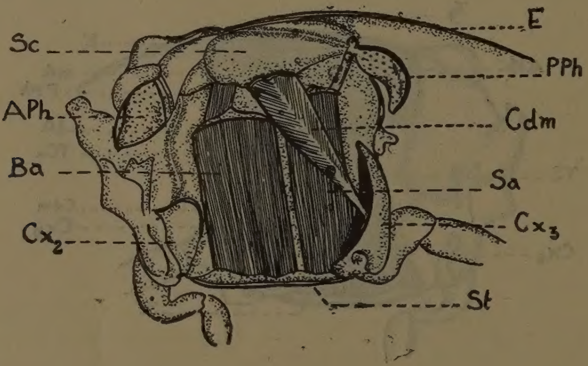

Fig. 2—Median lateral dissection of the metathorax of *Oryctes rhinoceros* showing the direct flight muscles.
The first set of median indirect flight muscles have been removed;
Ba—basalar muscle;
Sa—subalar muscle:
Sc—scutum;
Rest of the lettering as in Fig. 1.

The coxo-dorsalis metathoracis muscle (Fig. 1, & 2 Cdm.) provides the means whereby the coxa can be held firmly during flight. It is a muscle which originates on the scutum (Sc.) and is inserted on the dorsal rim of the coxa (Cx 3). It is situated between the two sets of indirect and direct muscles. At its origin on the scutum it is quite broad. Fibres are arranged in a pinnate fashion on either side of a central ribbon which is inserted on the dorasal rim of the coxa. This pinnate arrangement is clearly seen in *Oryctes rhinoceros.* Jackson (1956) has described this muscle in *Agabus sp.* (Dytiscidae) and also observed it in *Anacaena globulus* (Hydrophilidae), and many other species of *Dytiscus.* Recently we (1957) observed it in *Sternocera chrysis var. chrysidoides* (Buprestidae) and presently in *Hydrophilus olivaceous* (Hydrophilidae), *Anomala viridis* (Rutelini), *Platinotus punctatipennis* (Tenebrionidae), *Oryctes rhinoceros* (Scarabidae), *Acanthophorus serraticornis* (Cerambycidae), *Mylabris pustulata* (Meloidae) and one species belonging to the family Gyrindae. The muscle was however, found to be absent in *Cybister confusus* and *Cybister tripunctatus* (Dytiscidae) and *Agripnus fuscipes* (Elateridae). In these three forms and in *Sternocera chrysis* as well as in *Agabus* described by Jackson, the coxa is fused along both the dorsal and ventral rims and is immovable.

There can be little doubt that the muscle primarily was a coxal remotor. It is possibly homologous to the *third tergo-coxal* muscle of *Chortoicetes terminifera.* (Acridiidae., Orthoptera) described by Tiegs. (1955). Different functions have been attributed to the *coxo-dorsalis metathoracis* muscle. Bauer (1910) considered it a leg muscle, which due to the fixation of the coxa became functionless or perhaps functioned as a wing levator. In those forms in which the coxa is completely fused as for example in *Sternocera chrysis* and *Agabus sp.* it may function as a weak wing levator. But, its absence in forms like *Agrypnus fuscipes, Cybister tripunctatus* and *Cybister confusus* where also the coxa is immovably fixed, raises doubt on its having such a function. It may also be noted that the muscle is found to be smaller in size in Buprestidae where the coxa is fixed and flight very well developed. In Gyrinidae where the coxa is almost fused, like that of Dytiscidae, the muscle is highly reduced. Jackson's suggestion of its possible function in respiration, on the basis of its presence in terrestrial beetles, is however, doubtful. Its invariable presence in forms where the coxa is fixed only by the ventral rim and is consequently slightly movable, its smaller size in forms where the coxa is fused and its absence in some beetles in which the coxa is completely incorporated in the body wall, is not without significance. By its remotor action it can hold the coxa immovable during flight, thereby enabling the *tergo-coxal* muscles to function in flight. The tergo-coxals have lost the promotor action with the fusion of the rim of the coxa with the sternum, But their effective use as indirect wing muscles depend on the immovability of the entire coxa at the time of flight. This is achieved by the action of the coxo-dorsalis metathoracis muscle.

Summary

1. The presence of the coxo-dorsalis metathoracis muscle in *Sternocera chrysis*, *Hydrophilus olivaceous*, *Platinotus punctatipennis*, *Oryctes rhinoceros*, *Acanthophorus serraticornis*, *Mylabris pustulata* and a species of Gyrinidae and its absence in *Cybister confusus*, *Cybister tripunctatus* and *Agrypnus fuscipes* have been noted.
2. That the well developed condition of this muscle is correlated with the incomplete fusion of the coxa, has been brought to light.
3. Its possible function in holding the coxa immovable during flight thus enabling the tergo-coxal muscles to function as indirect flight muscles even without reversion of original insertion and attachments, has been suggested.

Acknowledgement

We wish to express our sincere thanks to Mr. D. R. Ranade of the Department of Zoology, University of Poona for the identification of beetles and also for offering valuable suggestions in writing this paper.

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OBSERVATIONS ON THE BEHAVIOUR OF *GREGARINA POECILOCERUM* UNDER HETEROSMOTIC CONDITIONS

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ALMOST all fresh water protozoans possess contractile vacuoles, whose rapid pulsation is an indication of a marked exchange of water with the surrounding medium. This is especially so in the ciliates. It is almost certain that in these cases the water excreted is not primarily metabolic in its origin but has either entered the body from the external medium by diffusion through the surface or has been taken in, in the formation of the food vacuoles.

Amongst the parasitic protozoa, contractile vacuoles are uncommon. While the parasitic falgellates and rhizopods rarely possess a contractile vacuole, the sporozoa never have one. Those sporozoa living in the guts of invertebrate hosts must naturally be experiencing wide variations in the osmotic pressure of their surrounding media and it is to be assumed that considerable exchange of water and salts between the interior of the parasite and the external medium takes place. But, with the lone exception of Adcock's (1940) study, no data in this regard are available. Extensive investigations are needed in this direction and in the following are presented some observations from studies still in progress.

Materials and Methods

Gregarines from the gut of the grass hopper, *Poecilocera picta*, were used in this study. The alimentary canal, of grass hoppers under chloroform anaesthesia, was opened and individual gregarines were transferred to 0.50 ml. of sodium chloride solution of the desired concentration. Tracings of the outline of the deutomerite were made at short intervals on millimeter graph paper, with the aid of the New Model Zeiss Camera Lucida. The latter projects the image on to the horizontal surface of the table and the inclined plane board is not necessary for drawing. Attention was confined only to the deutomerite for reasons best set forth in Adcock (1940). The area enclosed by the outline of the deutomerite as obtained on the graph paper was taken to be a function of thc volume of the cell. While such a method is admittedly not very refined, the remarkable reproducibility of results obtained using this method argues in favour of its dependability in the absence of anything better. Measurements were made on gregarines in different concentrations, ranging from 1.0 to 10.0 per cent of sodium chloride in distilled water.

Results

The changes in the area of the deutomerite of *Gregarina poecilocerum* in hypo-and hypertonic concentrations of sodium chloride solutions are typified by Fig. 1. It is seen that in all concentrations below 6.0 per cent, an increase in area is observed in all individuals studied, the time taken to reach the maximum value varying with the concentration of the external medium. The values begin to fall after a maximum and continue to fall till a value closely approximating the original value (the one at the

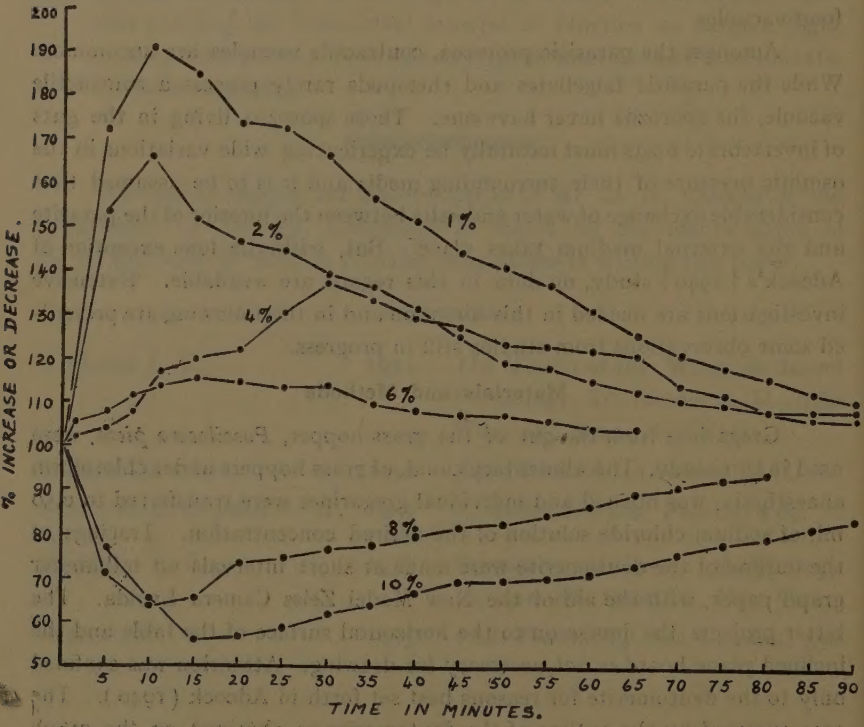

Fig. 1—Volume changes in individual gregarines kept in heterosmotic media. Each curve represents a single gregarine. Change in size is indicated as per cent of original value. Each point represents an actual single measurement.

start of the experiment) is reached after which they remain constant.

No significant volume changes were observed in gregarines kept in 6.5 per cent sodium chloride solution over extended periods (exceeding two hours).

When gregarines are transferred to sodium chloride solutions of concentration greater than 6.5 per cent there is a corresponding initial decrease in value, with subsequent return to the original size.

The maximum or minimum per cent of change in size is directly dependent on the concentration of the external medium (Fig. 2).

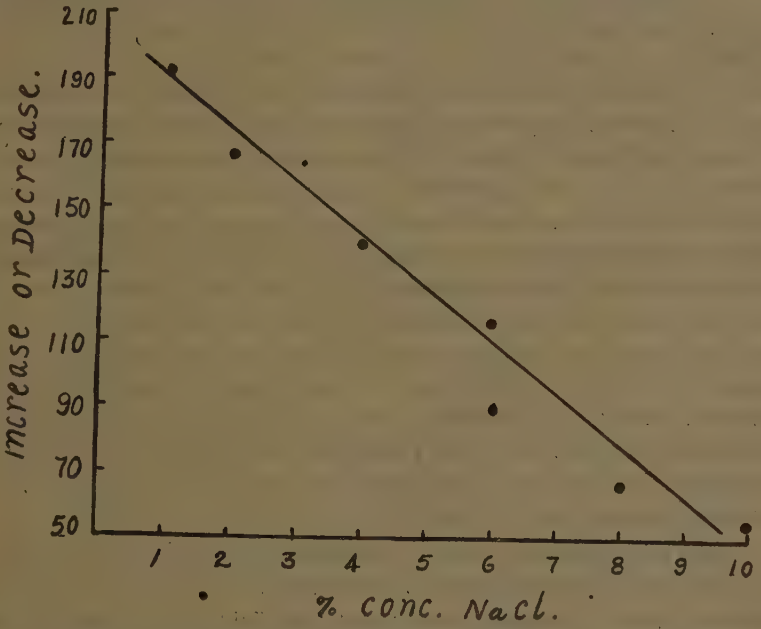

Fig. 2—The relation between the concentration of the medium and the maximum or minimum size attained. Values taken from Fig. 1.

Discussion

Adcock (1940) has observed that *Gregarina cuneata* rapidly reached a state of equilibrium in hyper—and hypotonic solutions of sucrose indicating absence of volume regulation. While Adcock's study was confined only to *G. cuneata*, Prosser (1950) referring to her work states that "*several* gregarines from the gut of meal worms swell and shrink according to the tonicity of the medium" (italics ours) meaning thereby that no volume regulation takes place in gregarines. It is to be admitted that Adcock's as well as the present studies show that the gregarines exhibit osmotic lability and hence are poikilosmotic, capable of only *adjusting* osmotically to changes in the medium. Osmotic change may involve exchange of water between the body and the medium resulting in volume change. But if salt is also exchanged, then the body volume tends to be kept constant in changing media. Many marine invertebrates such as *Aplysia* exhibit the latter phenomenon.

A similar behaviour, indicating volume regulation, is observed in *Gregarina poecilocerum*. It has been observed, and is shown in Fig. 1 that individuals of *G. poecilocerum* in hypotonic media swell, reaching a maximum and then gradually lose in volume till they approach their normal volume. Likewise in hypertonic solutions initial shrinking is followed by a return to the normal volume. This only implies a regulation of volume without any capacity to regulate the osmotic concentration of the internal medium. Such volume regulation as is exhibited in Fig. 1 is probably brought about passively, by allowing salts to reach a physical equilibrium with the gradient. This means that in hypotonic media there is an initial entry of water into the organisms, resulting in swelling. After the maximum volume is reached the loss of salts to the external medium reverses the flow of water across the membrance restoring the initial volume of the cell. In hypertonic media after an initial loss of water to the medium, salts and along with them water enter the living system and restore the initial volume. This volume control phenomenon indicates a permeability to salts in both directions.

Such a change in the osmotic concentration of the contents of the gregarine would naturally lead to a variable internal osmotic pressure depending upon the osmotic pressure of the gut fluid of the host. Hence it is to be expected that the isotonic value (6.5% NaCl) reported here would be subject to variation. Thus in an earlier series of experiments (Rao and Narasimhamurthi, 1955) it was noticed that a 4.75% sodium chloride solution was isotonic to the gregarine, as against the present value of 6.5%. It is likely that there will be seasonal or periodic changes in the osmotic pressure of these organisms depending upon the changes in the osmotic concentrations of the gut fluids of the host which in turn depend on the kind of food of the host and the availability of water to the host.

Summary

1. *Gregarina poecilocerum* swells in hypotonic media and shrinks in hypertonic media. But in both cases it returns to its original volume within a short period, thus exhibiting osmotic adjustment with volume regulation.
2. This capacity to adjust osmotically through volume regulation suggests a permeability to salts and water in both directions.

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VARIATIONS IN THE AMINO ACIDS OF LIVER OF *NOTOPTERUS NOTOPTERUS* (PALLAS) DURING STARVATION

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BIOCHEMICAL analysis of the liver of vertebrates shows that its major chemical compounds are glycogen, fat and complex proteins. But the former two are really tertiary energy stores of the body, which are drawn upon as occasion demands and during starvation. The fundamental structure of fat and glycogen is not altered, however much the animal is overfed or starved. But it is otherwise with proteins. These are continuously destroyed and resynthesised on a large scale in the liver cells and probably in other tissues too, as the classical work of Schoenheimer and his school has demonstrated.

In the normal animal the protein of the food is the source of nitrogen. The amino acid content of the liver may be as high as five times its normal resting value after a heavy protein meal. It has been shown in the rat that during the three or four hours ensuing a heavy protein meal the amino acid content of the liver drops, and simultaneously the amount of urea in the blood rises. When the diet with low protein content is taken, the amount of nitrogen excreted may be far greater than that ingested, the excess representing the nitrogen obtained by catabolism of the body proteins. But the investigations made so far have been almost exclusively on rat and a few other mammals. We have no knowledge of the state of affairs in cold-blooded animals, experiments on which are bound to be of great interest owing to their slow rate of metabolism.

I have been studying the physiology and biochemistry of the digestive system in the fresh water fish *Notopterus notopterus* (Pallas) and the present report deals with the changes in the free as well as protein-bound amino acids of the liver of specimens which had remained without food of any kind for seventy-five days. These specimens remained healthy and active and did not show any pathological symptoms. However, they showed a reduction in size and weight.

Material and Methods

A few specimens of *Notopterus notopterus* were kept alive in laboratory aquarium without food for seventy-five days. The liver was dissected out fresh and defatted by keeping it in acetone for two days. For quantitative study, the dry weight of the tissue was determined to the nearest milligram. For the preparation of free amino acid extract, 80% alcohol was used as the extractant. The material was ground up in a mortar with a minimum quantity of 80% alcohol and then centrifuged. To the supernatant liquid thrice its volume of chloroform was added, and centrifuged. The clear supernatant portion was taken for spotting. For the preparation of acid hydrolysates the method adopted by Giri et al. (1952 a, b) was followed. The weighed tissue was hydrolysed in 6N-HCl. under reflux for eighteen hours. The hydrolysate was evaporated to dryness over a water bath, and the acidity was removed by adding distilled water repeatedly. The dried residue was dissolved in 10% isopropanol, and the filtrate was used for spotting. For the circular paper chromatogram Whatman filter paper No. 1 with 13 cm. diameter was used and for two dimensional chromatogram paper with 20 × 20 cm. was used. The solvent system consisted of n-butanol, acetic acid and water mixed in the ratio of 4 : 1 : 1 : v/v and for two dimensional chromatography in addition to the above solvent buffered phenol was used. 0.5% ninhydrin in 95% acetone was used as the colour developing reagent. The amino acids were identified by running mixed chromatograms of known and unknown amino acids run under identical experimental conditions and with reference to the Rf values of the known amino acids. Multiple running was resorted to for the separation of overlapping amino acids. For the quantitative determination of amino acids the procedure adopted by Giri (1953) using copper sulphate was followed. The spots in the two dimensional chromatograms were cut and eluted in 8.0 c.c. of a solution containing 7.2 c.c. of 75% alcohol and 0.8 c.c. of 0.1% copper sulphate solution. The optical density of the solution was determined with Lumetron colorimeter 401A Model with 550 mμ filter.

Results

The liver of the normal specimen showed the presence of the following free amino acids: 1. Leucine and Isoleucine, 2. Valine, 3. Methionine, 4. Threonine, 5. Glycine, 6. Glutamic acid, 7. Alanine, 8. Serine,

9. Aspartic acid, 10. Histidine, 11. Arginine, 12. Asparagine, 13. Lysine, 14. Ornithine and 15. Cystine.

In the liver of the starved fish Serine, Ornithine and Cystine were lacking.

The following protein-bound amino acids were present in the liver of the normal specimen: 1. Leucine and Isoleucine, 2. Phenylalanine, 3. Valine, 4. Methionine, 5. Proline, 6. Tyrosine, 7. Alanine, 8. Thronine, 9. Glycine, 10. Serine, 11. Glutamic acid, 12. Aspartic acid, 13. Histidine, 14. Arginine, 15. Asparagine, 16. Ornithine, and 17. Cystine.

In the liver of the starved animal Ornithine was lacking.

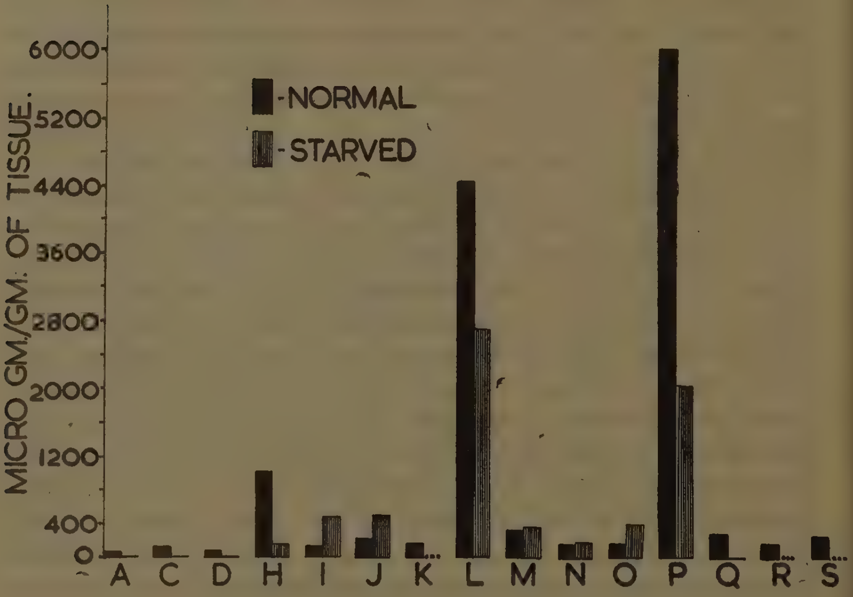
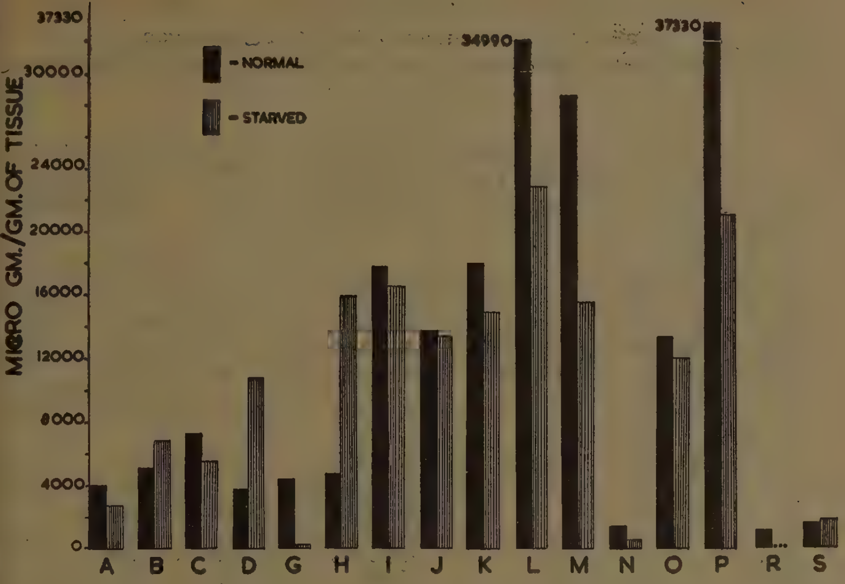


Fig. 1. Histogram of the free amino acids of the liver of normal and starved fish *Notopterus notopterus*.

—Represents the traces of amino acids.

..Represents the complete absence of the amino acids.

A. Leucines, B. Phenylalanine, C. Valine, D. Methionine, G. Tyrosine, H. Alanine, I. Threonine, J. Glycine, K. Serine, L. Glutamic acid, M. Aspartic acid, N. Histidine, O. Arginine, P. Asparagine, Q. Lysine, R. Ornithine and S. Cystine.



ig. 2 Histogram of the protein-bound amino acids of the liver of normal and starved fish *Notopterus notopterus.*

The quantitative differences were more striking than the qualitative differences. The histograms (Figs. 1 & 2) show the quantitative variation and the table below the percentage of reduction and of rise of the different amino acids in the liver during starvation. The overall reduction was greater in the case of free amino acids. Three amino acids showed cent percent reduction and four nearly cent percent reduction. Three free amino acids, Threonine, Glycine and Arginine, showed considerable increase and two free amino acids, Aspartic acid and Histidine about 10% rise. In the case of the protein-bound amino acids Ornithine showed cent percent reduction. Generally speaking the degree of reduction was less than in the case of free amino acids. Alanine and Methionine showed considerable rise,—over 200%—while Cystine only a slight rise of 1.8% and Phenylalanine, 35.7%.

It is evident that there are profound changes during starvation, in the protein-bound amino acids as well as in the metabolic pool of free amino acids. Further work is in progress.

Table showing quantitative changes in the free and protein-bound amino acids during starvation of *Notopterus*

	Amino acids which were completely absent	Those which showed nearly 100% fall		Those which showed a fall below 100%		Those which showed an increase in percentage	
Free amino acids	Serine	Leucines		Alanine	84.9	Threonine	344.0
	Ornithine	Valine		Asparagine	66.3	Glycine	122.7
	Cystine	Methionine		Glutamic acid	60.8	Arginine	122.5
		Lysine				Aspartic acid	11.0
						Histidine	10.0
Protein-bound amino acids	Ornithine	Tyrosine	96.0	Histidine	66.0	Alanine	239.0
				Aspartic acid	45.7	Methionine	273.9
				Asparagine	41.5	Phenylalanine	
				Glutamic acid	34.5		35.7
				Leucines	33.2	Cystine	1.8
				Valine	23.7		
				Serine	17.5		
				Arginine	10.5		
				Threonine	7.0		
				Glycine	2.9		

Summary

The effects of seventy-five days' starvation on the free and protein-bound amino acids of the liver of *Notopterus notopterus* (Pallas) have been investigated, using circular as well as two-dimensional paper chromatography.

Of the free amino acids normally present in the liver, serine, ornithine and cystine disappeared completely; leucine, valine, methionine and lysine were reduced to traces. Threonine, glycine and arginine showed considerable increase, and aspartic acid and histidine only a slight increase.

Of the protein-bound amino acids ornithine alone disappeared. Methionine and alanine increased considerably; phenylalanine increased slightly and cystine very slightly. The other amino acids showed varying degrees of reduction tyrosine (96%) and histidine (66%) at one extreme, and arginine (10.5%), threonine (7.5%) and glycine (2.5%) at the other.

Acknowledgement

I am greatly indebted to Prof. R. V. Seshaiya, Director, Marine iological Station, Portonovo, for suggesting the problem, guidance and elp.

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AN ELECTROPHORETIC STUDY OF PLASMA PROTEINS IN DISEASES

M. L. Pai

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A study on the plasma protein fractions in oedema and ascites was reported by the author (Pai, 1953). In the present study, the results of the investigations on plasma protein fractions by paper electrophoresis in certain diseases, are reported.

Materials and Methods

Twenty eight cases admitted in the wards of the S. S. G. Hospital, Baroda, for the conditions of hypoproteinemia, have been studied for their plasma protein fractions. The total protein content was determined by the micro-Kjeldahl method of King (1946), in the portion of the plasma obtained from the oxalated blood collected in the morning by venepuncture. The fractionation of the proteins in an aliquot sample of the plasma obtained as above, was done by paper electrophoresis. After the preliminary treatment of the filter paper strip for equilibration for ten minutes, unhaemolysed plasma was applied, in quantity of 0.005 ml. by means of a capillary delivery micro-pipette, to the filter paper strip at a distance of 2 cm. from the cathode end. The veronol buffer of pH 8.6 and ionic strength 0.05 was used. Six to eight strips were run simultaneously in two cabinets, for 16 hours at 110 to 115 Volts and at about 5 milliamperes current. The strips were dried and stained subsequently in 1 per cent Amido Black 10 B (Aniline Schwarz 10 B) in 9:1 methanol glacial acetic acid mixture, using glass-cylinders with an arrangement of circulation of an air current. The excess of dye was removed by means of a wash liquid, with successive washings. After drying these strips in air, five definite fractions could be delineated visually. Each fraction was quantitatively determined by plotting on graph paper, readings obtained for the optical density on a Densitometer and then by computing the product of the proportionate area covered by the fraction in the curve plotted and the total protein content in proportion of the whole area of the curve. The results obtained are shown in the table and are represented in a diagrammatic curve. (Fig. 1).

Results

Table showing the average plasma proteins in diseases studied by paper electrophoresis

Sr. No.	Condition of the patients	No. of cases studied.	Total Protein	Plasma Proteins in gms. %				
				Albumin	α_1 Globulin	α_2 Globulin	β Globulin	Gamma Globulin and fibrinogen
1	Cirrhosis of liver	8	5.92	2.18	0.48	0.67	0.81	1.83
2	Infective Jaundice ..	5	5.09	1.74	0.25	0.59	0.63	1.81
3	Hypoproteinaemia ..	4	6.33	2.14	0.56	0.80	0.79	1.56
4	Nephritis	6	5.68	1.71	0.19	0.73	0.96	2.08
5	Osteomalacia	5	7.22	2.55	0.47	0.87	0.96	2.35

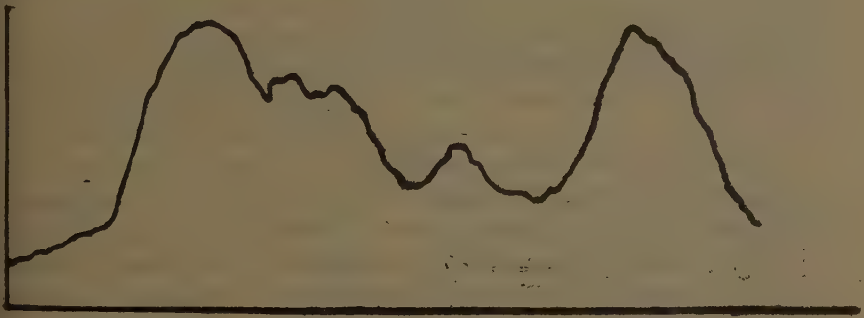

Fig. 1. Nature of the curve obtained after plotting the readings obtained on the densitometer for optical density of the plasma protein fractions separated electrophoretically, in a case of hypoproteinemia. Five peaks obtained are albumin, α_1 globulin α_2 globulin, β-globulin and gammaglobulin and fibrinogen respectively, as read in order from the right-hand end.

Discussion

The nature and composition of the plasma proteins are becoming increasingly important in clinical diagnosis. Many physical and chemical methods have been in use to accomplish the fractionation of blood proteins, but with the recent introduction of the electrophoretic technique many of the difficulties encountered in the above methods have been eliminated. This method therefore has become the most important tool in clinical protein research. Work on the electrophoretic study of plasma proteins in certain diseases was undertaken, the results of which are given here. Out of twenty two cases, eight cases were of cirrhosis of liver, three of infective type of jaundice, four hypoproteinemic, two with nephritic condition and five diagnosed as osteomalacia.

From the table it will be seen that in all the conditions except osteomalacia, the total proteins in plasma were lower than the normal values. The reduction in the total proteins was mainly due to the reduction in the plasma albumin values. In osteomalacia the reduction in the total plasma porteins is not much lower than the normal values. The average values obtained for total plasma proteins in osteomalacia are 7.22 gm.%, whereas for the other conditions studied, the corresponding values ranged between 5.09 and 6.33 gm.%. The average values for plasma albumin concentration in these conditions ranged between 1.71 and 2.18 gm.%. The corresponding average value in osteomalacia was 2.55 gm.%, which was thus a little more than the range obtained in other conditions. The other fractions of plasma proteins in all these conditions studied did not deviate much from the normal values. The normal values obtained in Indians have been given as 7.29 gm.% total plasma proteins and 4.47 gm.% plasma albumin (Kulkarni *et al*, 1958); and 7.70 gm.% total plasma proteins and 4.52 gm.% plasma albumin (Satoskar *et al*, 1954). In the previous communication made from this laboratory total plasma proteins and plasma albumin values obtained in conditions of oedema and ascites were also found to be both reduced below the normal values. The estimations of both the total plasma proteins and the fractionation of proteins in the previous communication were made by chemical methods, whereas in the present investigation the total plasma proteins were estimated by chemical methods and the fractionation of the proteins obtained by paper electrophoresis.

Similar findings showing that there is reduction in both total plasma proteins and plasma albumin levels in various conditions have been reported by several workers. Patel *et al* (1957) reported that total plasma proteins and albumin are lower in malnutrition and lowest in the oedema groups. Satoskar *et al* (1954) found that there was a drop in the albumin fraction in cases of virus hepatitis.

Summary and Conclusions

(1) Twenty eight cases admitted in the wards of S. S. G. Hospital, Baroda, for conditions like cirrhosis of liver, infective type of jaundice etc. have been studied quantitatively for total plasma proteins, the fractionation of proteins having been obtained by paper electrophoresis.

(2) Average plasma protein values ranged from 5.09 to 6.33 gm.% in conditions other than osteomalacia, whereas the range for the plasma albumin levels was from 1.71 to 2.18 gm.%. In osteomalacia the corresponding values were 7.22 gm.% and 2.55 gm.% respectively, which were thus higher than those obtained in other conditions. The total plasma protein levels as well as the plasma albumin levels therefore seemed to be lower than the normal values.

(3) The findings have been compared with those of other workers and discussed.

Acknowledgement

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A STUDY OF THE PLASMA PROTEINS OF SOME REPTILES AND MAMMALS

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HOWELL (1922) pointed out that the albumin present in the plasma of cold-blooded vertebrates is surprisingly low, compared to mammals. Howe (1925) giving the plasma protein values for a number of animals stated that environmental factors might produce fluctuations in one fraction of plasma proteins, without marked changes in the others. Redfield (1933) after a thorough study of the then available plasma protein values for a number of animals came to the conclusion that in the course of evolution of blood, the plasma proteins indicate a progressive quantitative increase, in the evolutionary scale, from invertebrates to lower and higher vertebrates. Lusting and Ernst (1937) found out the amount of plasma proteins in a number of vertebrates and invertebrates. Besides these, many others have provided much material data on the plasma proteins of various animals. Florkin *et al.*, (1949) state that the protein concentration of the plasma is " incontestably correlated with the position occupied by the animal, in the scale of zoological classification. " Redfield (1933) attributed this to the more highly developed and efficient cardiovascular system, capable of circulating blood at a greater pressure, in the higher animals. Menon (1952) working on representative types from each class of vertebrates, supported this conclusion of Redfield of an increase or decrease in the amount of plasma proteins, depending on the evolutionary status of the animal. He also found that the relative proportion of albumin to globulins shows a marked increase, from the lower to the higher vertebrates.

In the light of the above observations it was expected, that the determination of total proteins and concentration of the different proteins in the plasma of more animals may throw further light, on their biochemical evolution as much as their adaptation to a particular environment and mode of life.

Material and Methods

In the present investigation the plasma proteins of the following reptiles and mammals were studied :

Reptiles

1. *Varanus griseus*
2. *Ptyas mucosus*
3. *Naja naja*
4. *Vipera russelli*
5. *Distira eyanocincta*
6. *Natrix piscator*
7. *Lissemys punctata*
8. *Geomyda triguga*

Mammals

1. *Suncus caeruleus*
2. *Felis domesticus*
3. *Pteropus giganteus*
4. *Macaca radiata*

The plasma for analysis was obtained by centrifuging oxalated blood. In the determination of the total proteins, albumin and fibrinogen of the plasma, the micro-Kjeldahl methods given by Hawk *et al* (1949) which are combined adaptations of the procedures of various authors, have been adopted. For making the appropriate correction for non-protein nitrogen in the estimation of total proteins and albumin, the non-protein nitrogen of plasma was determined by the method of Folin and Wu as given by Hawk *et al* (1949). The amount of globulins was determined by substracting the total amount of albumin and fibrinogen from the total protein value. All readings were taken in the Klett-Summerson photo-electric colorimeter. Results are tabulated in Table 1.

Results and Discussion

After a study of the total protein values in the plasma for a number of vertebrates from fishes to mammals Redfield (1933) arrived at the conclusion that there is an increase in the concentration of proteins in the plasma, as we go higher up in the evolutionary series. He also indicated that the increase in the plasma protein values is " paralled with the development of a cardiovascular system capable of containing blood at increasing pressures ". According to this statement there should be a higher percentage of total proteins in the plasma of mammals than in that of reptiles. This conclusion stands supported by my data except for that of Ptyas. The value obtained for Ptyas seems to be quite abnormal as it surpasses all other values obtained both for reptiles and mammals. The figure for Ptyas shows that there is the probability of evolution resulting in a higher level in this respect within reptiles too.

A close examination of the total protein values of the plasma of the reptiles investigated shows a comparatively high value for the uricotelic

TABLE I

Total proteins, albumin, globulin and fibrinogen contents of the blood of the animals investigated and the albumin/globulin ratio calculated from these figures

Name of the animal	Total protein values g./100 ml. of plasma	Amount of albumin in g./100 ml. of plasma	Amount of globulins in g./100 ml. of plasma	Albumin/ globulin ratio	Fibrinogen content of the plasma in g./100 ml.
Mammals					
Macaca	7.139±0.766	3.366±0.391	3.617±0.478	1.027±0.170	0.209±0.042
Felis	6.793 ,, 1.385	3.523 ,, 0.749	3.136 ,, 0.876	1.054 ,, 0.351	0.306 ,, 0.044
Pteropus	6.435 ,, 0.297	3.367 ,, 0.195	2.581 ,, 0.511	1.372 ,, 0.346	0.514 ,, 0.086
Suncus	6.145 ,, 1.069	2.895 ,, 0.602	2.867 ,, 0.899	1.260 ,, 0.516	0.586 ,, 0.023
Reptiles					
Varanus	5.603 ,, 0.551	1.862 ,, 0.353	3.262 ,, 0.772	0.629 ,, 0.255	0.373 ,, 0.053
Lissemys (Serum used)	3.339 ,, 0.224	0.781 ,, 0.096	2.559 ,, 0.129	0.304 ,, 0.023	0.500 ,, (Shah)
Geomyda	4.133 ,, 0.667	0.737 ,, 0.106	3.124 ,, 0.721	0.242 ,, 0.078	0.316 ,, 0.022
Ptyas	11.219 ,, 3.954	3.414 ,, 1.544	7.635 ,, 2.388	0.416 ,, 0.080	0.170 ,, 0.022
Vipera	5.085 ,, 0.070	2.321 ,, 0.349	2.649 ,, 0.361	0.916 ,, 0.252	0.153 ,, 0.006
Naja	5.236 ,, 0.955	2.622 ,, 0.161	2.535 ,, 0.807	1.152 ,, 0.325	0.084 ,, 0.027
Distira	4.508 ,, 0.704	0.962 ,, 0.202	3.523 ,, 0.689	0.260 ,, 0.069	0.136 ,, 0.046
Natrix	5.064 ,, 0.344	1.520 ,, 0.607	3.453 ,, 0.326	0.463 ,, 0.218	0.138 ,, 0.035

forms, Varanus, Natrix, Ptyas, Naja and Vipera in contrast to the chelonians which are ureotelic.

Reviewing the total protein values obtained for reptiles and mammals, it is seen that the reptiles exhibit a greater range of variation among themselves than the mammals. Thus among the reptiles the total protein value ranges from 3.339 ± 0.224 in Lissemys to 11.219 ± 3.954 in Ptyas, while in mammals the variation is only from 6.145 ± 1.069 in Suncus to 7.139 ± 0.766 in Macaca.

Regarding albumin, globulins and albumin/globulin ratio of the plasma of reptiles very little information is available from previous work. Menon (1952) obtained a value of 1.480 to 1.519 for albumin content, 1.3822 to 1.1652 for globulin and an albumin/globulin ratio of 1 to 15 for Uromastix. His values compare favourably with those obtained by me for Varanus. Howell (1922) mentioned that cold blooded vertebrates are very poor in the albumin content of the plasma which at times is as low as 0.36 to 0.69. An examination of the albumin content and the albumin/globulin ratio of the various animals investigated shows that, by far in the vast majority of cases the albumin content as well as the albumin/globulin ratio are high in mammals compared to those in reptiles. Menon (1952) after studying the albumin content and the albumin/globulin ratio of representative species from each class of vertebrates arrived at the conclusion that even though there is a rise in the total protein content in the plasma, as we go higher up in the evolutionary series there is no corresponding increase in the globulin content of the plasma, while the albumin content shows marked increase. But the fact that at least one reptile (Naja) has an albumin/globulin ratio equal to that of a mammal (Macaca) shows that even among reptiles evolution may result in producing types with increased albumin/globulin ratios. This partiality for albumin is probably due to its capacity to impart greater colloid osmotic pressure to the plasma compared to the globulins, by virtue of its low molecular weight. This is of great advantage to an animal maintaining a high blood pressure in its circulatory system. Prosser *et al* (1950) have given values for blood pressure in mammals ranging from 90 to 194 mm. of mercury, while the corresponding figures for reptiles (crocodiles and turtles) given by him range from 18 to 50 mm. of Hg. Without obtaining blood pressure values for animals like Varanus and Naja with higher albumin values, one hesitates to hold the view that the blood pressure values for reptiles are lower than that of mammals.

The fibrinogen content in Lissemys could not be estimated as the blood invariably laked as soon as it was mixed with potassium oxalate. A similar laking of the blood of the turtle *Chelydra serpentina* on the removal of calcium ions from the blood was reported by Wingfield *et al* (1949). Shah (1956) who used heparinised blood in his studies on reptilian blood has recorded a fibrinogen content of 0.500 gm./100 cc of plasma, for Lissemys.

The fibrinogen content of the blood of reptiles and mammals shows marked variations. Thus among mammals the values obtained for Suncus and Pteropus are high compared with those of Macaca and Felis. Among reptiles, the snakes have surprisingly low values while Varanus and Geomyda show comparatively high values. Values obtained by Shah (1956) for Chelonians are higher than those of other reptiles and some mammals. According to Scheer (1948) the fibrinogen content of many of the elasmobranchs, are very low and mammals have the highest values, while teleosts, reptiles and birds have intermediate amounts. But judging from the large number of cases available both from previous work as well as from the present study, where some mammals have lower values than certain reptiles, a general conclusion that the fibrinogen content of the blood in mammals is greater than in reptiles seems untenable.

Summary

1. Mammals show a greater concentration of proteins in the plasma than many reptiles.
2. Reptiles exhibit a greater range of variation in the total proteins compared to mammals.
3. Though mammals seem to have a greater amount of albumin than globulins compared to reptiles, at least one case (Naja) shows that even a reptile can have a higher albumin/globulin ratio.
4. The fibrinogen content of the blood of snakes seem to be very low.
5. Judging from the many values available for the fibrinogen content of the plasma of mammals and reptiles, the belief that mammals have a greater amount of fibrinogen in the blood than reptiles seems untenable.

Acknowledgement

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HISTOCHEMICAL DEMONSTRATION OF CERTAIN ENZYMES IN THE ADIPOSE TISSUE OF THE FOWL (*GALLUS DOMESTICUS*) AND ROSY PASTOR (*PASTOR ROSEUS*)

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(1 Plate)

IT has been shown that fat is the main source of energy for long and sustained muscular activity (Weis Fogh, 1952; Bing, 1954; George and Jyoti, 1957). Fat is stored in muscle itself and also various other tissues. In adipose tissue fat is stored in large amounts and is probably transported to sites where it is utilized during activity. Birds store large amounts of fat in their adipose tissue found at many sites in the body. Migratory birds especially accumulate large amounts of fat in their adipose tissue during the premigratory period (McGreal and Farner, 1956; King and Farner, 1956; Odum and Connell, 1956) and much of this appears to be used up during migratory flight. Synthesis of fat takes place in various tissues. Liver is the organ well-known to synthesize large amounts of fat. Adipose tissue can synthesize fatty acids at no lesser rate than liver when reduced to cytoplasmic unit namely fat free tissue or total nitrogen content (Feller, 1954). These and also the experiments of various others (Hausberger *et al,* 1954; Schoenheimer, 1942—to cite only a few) have confirmed that adipose tissue is a metabolically active tissue.

In an earlier paper we (1958 a) reported the activity of certain enzymes in the pigeon adipose tissue. Quantitative and histochemical studies on lipase activity revealed a very high concentration of this enzyme. Alkaline phosphatase, succinic dehydrogenases and certain other dehydrogenases were also found to be present in considerable amounts. In the light of these observations on the adipose tissue of the pigeon we suggested a high metabolic activity for this tissue. The pigeon is known to be a good flier indulging many a time in sustained flight.

In the present paper is reported some histochemical observations on certain enzymes of the adipose tissue of the rosy pastor (*Pastor roseus*) and the fowl *(Gallus domesticus)*. The following enzymes were studied with a view to comparing the relative activities of this tissue in the two birds :—lipase, alkaline phosphatase and succinic dehydrogenase.

Material and Methods

Fowls used in this study were the domesticated Indian indigenous ones. Only roosters were used in all cases. Rosy pastors were shot and tissues taken immediately. In both the cases after decapitation the visceral adipose tissue (from the ventral side, covering the coils of the intestine) was taken after most of the blood was drained off. Fresh frozen sections of this tissue was used throughout. The enzymes were studied histochemically according to the methods adopted in our earlier paper (1958 a).

Results

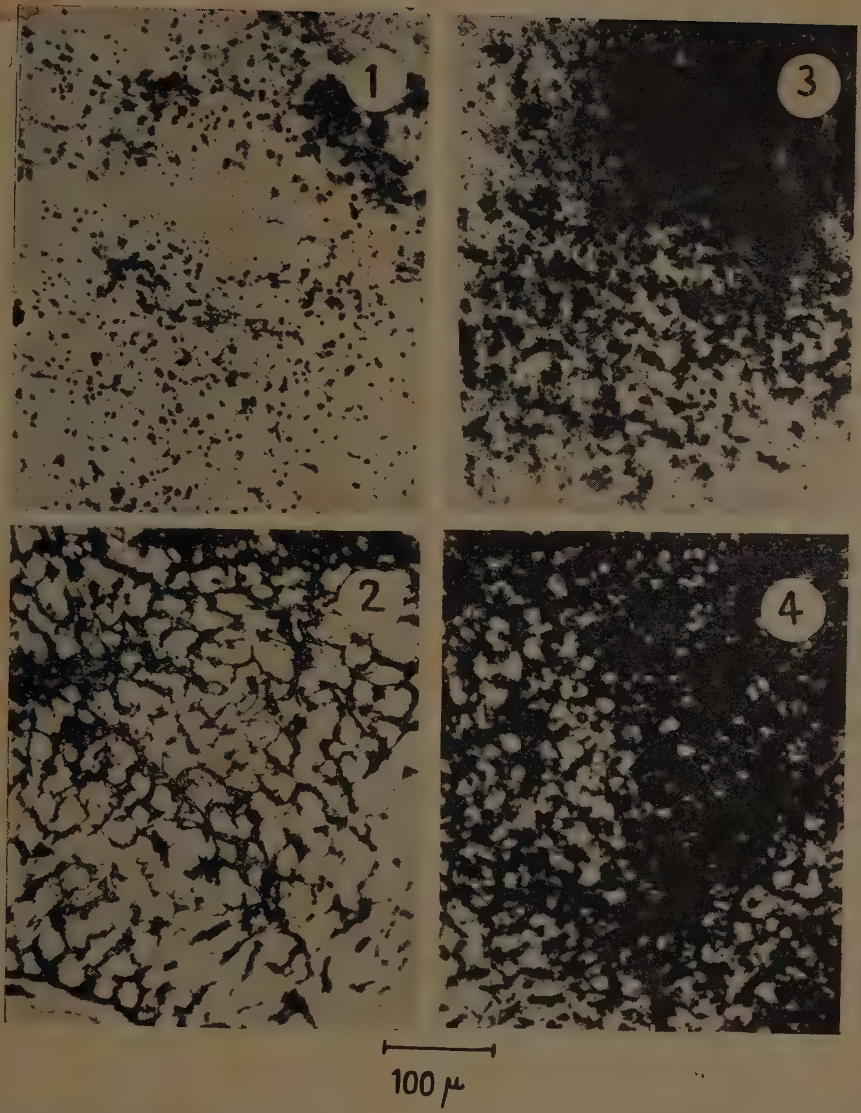
Lipase: Figures 1 and 3 present the photomicrographs of sections of adipose tissue of fowl and rosy pastor respectively treated to show lipase activity. The adipose tissue of rosy pastor showed a much higher activity as is evident from the more abundant deposit of the precipitate.

Alkaline phosphatase: The adipose tissue of rosy pastor showed a much higher activity than the same tissue in fowl. Figures 2 and 4 present the photomicrographs of sections of fowl and rosy pastor adipose tissue respectively treated to show alkaline phosphatase activity. Cell periphery is conspicuous by its dark colour indicating high degree of activity, since the cytoplasm of the cells is confined to the boundary while the inside is filled with fat. In the case of the rosy pastor the capillary endothelium too stands out dark due to the high activity of the enzyme. As far as fowl is concerned such pronounced activity could not be detected. This is probably because the capillary density may be less in the adipose tissue of the fowl compared to that of the rosy pastor or else the over-all activity of the tissue is less in the fowl.

Succinic dehydrogenase: The activity of this enzyme was almost the same in both rosy pastor and fowl, perhaps a little more in the former.

Discussion

The adipose tissue of birds and probably many other animals appears to play a major role in the lipid metabolism of the body. Enormous quantities of fat are stored in the adipose tissue of migratory birds prior



EXPLANATION OF FIGURES

Figures 1 and 3.—Photomicrographs of sections of adipose tissue of fowl and rosy pastor respectively treated for lipase activity. Gomori's "Tween" method.

Figures 2 and 4.—Photomicrographs of sections of adipose tissue of fowl and rosy pastor respectively treated for alkaline phosphatase activity. Gomori's method.

to migration. The recent studies conducted in our laboratories on the flight muscles of birds tend to show that fat is chiefly utilized for muscular energy during their migratory flight. Further, there is evidence to show that adipose tissue is capable of actively synthesizing fatty acids (Feller, 1954 ; Hausberger *et al*, 1954). Feller (1954) showed incorporation of acetate carbon into lipides of adipose tissue slices using C^{14} H_3 COONa. Hausberger *et al* (1954) used C^{14} glucose and showed synthesis of fatty acids from glucose. Both authors worked on rat adipose tissue and compared the activity with that of liver of the same animals. They found that when calculated for fat-free tissue or total nitrogen, fatty acid synthesis in adipose tissue is equal to if not more than that in the liver. The conversion of fatty acids thus formed into fat should be carried out by the high concentration of the lipase present in this tissue. Adipose tissue can thus not only synthesize and store large amounts of fat but also release them into the metabolic pool. Schoenheimer (1946) using isotopes as tracers has demonstrated that the fat in the adipose tissue is not a static reserve but is very much in a dynamic state.

Succinic dehydrogenase has been shown to be present in the adipose tissue of rat (Fawcett, 1952), guinea pig (Menschik, 1953), bat (George and Eapen, 1958 b) and pigeon (George and Eapen, 1958 a). The presence of this enzyme provides evidence to show that Krebs Cycle operates in this tissue.

Fawcett (1952) has reported the presence of alkaline phosphatase in the adipose tissue of rat and we have reported the presence of the same enzyme in the adipose tissue of pigeon (1958 a) and of bat (1958 b). With regard to this enzyme as well as lipase, rosy pastor and fowl showed a marked difference in activity. The higher concentration of both these enzymes in rosy pastor adipose tissue is an indication of its higher activity. It is possible that this is dependent on the difference in the general activity of these two animals. The rosy pastor is a migrant migrating from India to Europe by the end of April or the beginning of May and returning by August. Rosy pastors utilized in these studies were shot approximately two weeks before they disappeared from Baroda. At this time the adipose tissue is very active and large quantities of fat accumulate in them. There is a marked increase in the amount of fat deposited in the adipose tissue of rosy pastors from about 30 days prior to their migration. By the time they begin to leave from this locality the fat depots were found to be virtually filled with fat. In sharp contrast the fowl is known to be a very poor flier and consequently the general body metabolism is also low. In conclusion it can be said that the over-all activity of the adipose tissue of rosy pastor during the later part of the premigratory phase is very much higher than that of the same tissue in the fowl.

Summary

1. The activity of lipase, alkaline phosphatase and succinic dehydrogenase in the adipose tissue of fowl and rosy pastor was studied by histochemical methods.

2. Lipase and alkaline phosphatase were found to be much more in the rosy pastor adipose tissue than in that of fowl. No appreciable difference in the activity of succinic dehydrogenase could be detected. The significance of these observations is discussed.

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PROTEIN-BOUND AMINO ACIDS OF THE PITUITARY GLAND IN VERTEBRATE TYPES

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ROBERTS *et al* (1957) have pointed out that the distribution of free or loosely bound amino acids in each normal animal tissue in the corresponding stages of development in different species is characteristic for each species. In a recent communication (1957) they have illustrated this species specificity of the amino acid distribution of tissues with reference to the heart muscle of different vertebrate types.

I have been studying the amino acids, protein-bound as well as free, in the pituitary of different vertebrates. The present communication describes the patterns of protein-bound amino acids found in the pituitary in the sexually mature stage in different vertebrate types *viz. Labeo fimbriatus* (Bloch), *Mystus seenghala* (Sykes), *Boleophthalmus boddaerti* (Day), *Rana hexadactyla* (Lesson), *Calotes versicolor* (Daud), *Gallus domesticus* and *Rattus rattus*.

Material and Methods

The pituitary glands from fresh specimens were dissected out and placed immediately in 6N HCl. In view of the minute size of the gland in most species, the entire gland was taken for analysis. Acid hydrolysis in 6N HCl was conducted under reflux for 18 hours. At the end of the hydrolysis period the products of the hydrolysis in the test tube were concentrated to dryness on a steam bath. A few drops of 10% Isopropyl alcohol was added to the residue as a preservative and the extract was used for spotting.

Circular chromatography as well as ascending two-dimensional chromatography were carried out using Whatman Filter Paper No. 1. For circular chromatography filter paper with 13 cm. diameter was employed and for the two-dimensional chromatography 25 × 25 cms. and 20 × 20 cms. were used. Butanol-acetic acid-water mixture (4 : 1 : 1) and buffered phenol were used as solvents. 0.1% ninhydrin in 95% acetone was used as the colour developing reagent. The amino acids were identified from their positions in the standard chromatogram and from the R_f values previously calculated for known amino acids.

Observations

The qualitative distribution of the protein-bound amino acids in the pituitary gland of the different vertebrate types is shown in the Table I. It is seen that each of the species studied has its own distinctive pattern of protein-bound amino acids in the pituitary.

Eighteen amino acids were identified in all in the pituitary hydrolysates. The most important findings were as follows:

Leucine and Isoleucine, Phenylalanine, Alanine, Serine Glutamic acid and Arginine are found in the pituitary gland in all the forms studied.

(1) The pituitary of *Mystus seenghala* is different from other types in that it lacks only Cystine. (2) *Boleophthalmus boddaerti* differs from other types in that Valine and Methionine are wanting, in addition to Cystine. (3) *Labeo fimbriatus* lacks Asparagine, Lysine and Tyrosine besides Cystine. (4) *Rana hexadactyla* differs from all the other types studied in lacking Histidine, Proline and Threonine. (5) *Calotes* and *Rattus* have Cystine but can be distinguished from each other by the presence of Aspartic acid in the latter but not in former. (6) The bird differs from all the other types studied in lacking Glycine. (7) Lysine and Ornithine are present only in *Mystus* and *Boleophthalmus* but not in others.

From the Table it can be seen that the pituitary of each type studied differs from others by the presence or absence of one or more amino acids. Available information on comparative biochemistry of the pituitary hormones suggests marked differences in the chemical constitution among the different vertebrates (Hoar, 1957). The distinctiveness of the protein-bound amino acids in the pituitary of each of the species studied shows that their pituitary glands have distinctive protein specificity. In other words the biochemical basis of the secretion of the pituitary in the different species studied differs with each species.

Summary

The protein-bound amino acids of the pituitary glands of the sexually mature specimens of *Labeo fimbriatus* (Bloch), *Mystus seenghala* (Sykes), *Boleophthalmus boddaerti* (Day), *Rana hexadactyla* (Lesson), *Calotes versi-*

TABLE I

No.	Name of the species	Leucine & Isoleucine	Phenylalanine	Valine	Methionine	Proline	Tyrosine	Alanine	Threonine	Glycine	Serine	Glutamic acid	Aspartic acid	Histidine	Arginine	Asparagine	Lycine	Ornithine	Cystine
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
	Fishes																		
1	*Labeo fimbriatus*	A	B	—	—	E	—	G	H	I	J	K	L	M	N	—	—	—	—
2	*Mystus Seenghala*	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	—
3	*Boleophthalmus boddaerti*	A	B	—	—	E	F	G	H	I	J	K	L	M	N	O	P	Q	—
	Amphibia																		
4	*Rana hexadactyla*	A	B	C	D	—	—	G	—	I	J	K	L	—	N	O	—	—	—
	Reptile																		
5	*Calotes versicolor*	A	B	C	—	E	F	G	H	I	J	K	—	M	N	O	—	—	R
	Bird																		
6	*Gallus domesticus*	A	B	C	—	E	—	G	H	—	J	K	L	M	N	—	—	—	—
	Mammal																		
7	*Rattus rattus*	A	B	C	—	E	F	G	H	I	J	K	L	M	N	O	—	—	—

(For convenience of ready reference the amino acids have been indicated by the letters of the alphabet.)

Table showing the distribution of Protein-bound amino acids in the pituitary of vertebrate types.

color (Daud), *Gallus domesticus* and *Rattus rattus* have been determined chromatographically. It was found that the pattern of the protein-bound amino acids in the pituitary is distinctive of each of the species studied, which indicates that the pituitary glands have protein specificity.

Acknowledgement

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STUDIES ON THE STRUCTURE AND PHYSIOLOGY OF THE FLIGHT MUSCLES OF BATS

3. Alkaline Phosphatase and Succinic Dehydrogenase Activity in the Breast Muscle—A Histochemical Study

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It has been reported that the breast muscle of the pigeon (George and Naik, 1958 a and b) and bat (George and Naik, 1957) contains two types of fibres, a glycogen loaded white variety with few or no mitochondria and a fat loaded red veriety having a large number of mitochondria in them. George and Scaria (1958 a) and George, Susheela and Scaria (1958) from a histochemical study of lipase activity in these muscles concluded that the white fibres do not contain lipase. It was also observed that in the pigeon breast muscle only the border of the white fibres shows a positive staining reaction when tested for alkaline phosphatase according to the Gomori calcium cobalt method, whereas a uniformly deep staining was obtained in the red fibres (George, Nair and Scaria, 1958). Recently, George and Scaria (1958 b) studying the dehydrogenase activity in the pigeon breast muscle and certain other vertebrate skeletal muscles found that all the muscles fibres whether red or white with the exception of the white fibres of the pigeon breast muscle contain the Krebs cycle enzymes in appreciable concentrations and in the white fibers the concentration of these enzymes is less as compared to the red fibres. It was therefore thought worthwhile studying the alkaline phosphatase and succinic dehydrogenase activity in the breast muscle of the bat to find out how far the red and the white fibres in this muscle compared with those in the breast muscle of the pigeon.

Material and Methods

Transverse sections of the *pectoralis major* muscle of the Rhinolophid bat (*Hipposideros speoris*) were used for the study. For alkaline phosphatase, frozen sections cut according to the method of George and Scaria (1958 a) were dried at room temperature on clean slides, fixed in 10%

formalin at 4°C for 2 hours, washed in running water for 30 minutes, rinced in distilled water and incubated in the substrate medium for 24 hours at 40°C. The substrate medium was prepared according to Gomori (Pearse, 1954) and contained sodium glycerophosphate as substrate. The sections were then treated with cobalt nitrate and ammonium sulphide respectively. Boiled sections were used as control. In another control, the sections after fixing were treated with cobalt nitrate and ammonium sulphide before incubation.

Succinic dehydrogenase was studied according to the method of Straus, Cheronis and Straus (George and Scaria, 1958 b) using 2 : 3 : 5 triphenyltetrazolium chloride (TTC) as the dye. Thin frozen sections were cut and transferred to cold 0.1M phosphate buffer of pH 7.2. After ten minutes they were immersed in the incubation medium in a cuevette, covered with lid and incubated for about 15 minutes at 37°C, fixed in 10% neutral formalin, washed in water and mounted in glycerine jelly without counterstaining. The incubation medium contained the following in a total volume of 2.5 ml : 1.5 ml. 0.1 M phosphate buffer of pH 7.2, 1 ml. 0.1 M sodium succinate and 7.5 mg. TTC.

Results and Discussion

It was possible to distinguish more than two types of fibres in the breast muscle of the bat as judged from the degree of enzyme activity and the number of mitochondria as revealed by the formazan. The narrow red fibres showed the maximum activity of succinic dehydrogenase and the maximum number of mitochondria and some of the broad white fibres the minimum. Fibres which were intermediate in diameter between the broad and narrow ones showed varying degrees of activity depending on the diameter of the fibres. The mitochondrial density of the fibres also varied according to the diameter. Thus there was found to be a direct and clear cut correlation between the diameter and mitochondrial density of the fibres and their enzyme activity. A similar observation was made by George and Scaria (1958 b) in the leg muscle of the fowl. But the breast muscle of the bat differs from the leg muscle of the fowl in that the bulk of the former is made up mostly of narrow fibres, a feature naturally associated with greater activity, and the broader fibres are, much less in number and occupy the superficial region of the muscle (George and Naik, 1957). Further evidence that the enzyme activity in the muscle fibres is related to the size of the fibres is seen in the recent work of Nachmias and Padykula (1958). It should be pointed out that the fibres in the breast muscle of the bat which do not show the presence of fat (George and Naik, 1957) or lipase (George, Susheela and Scaria, 1958) are really the broad fibres which show the minimum activity of succinic dehydrogenase.

Alkaline phosphatase was found to be present in all the fibres. But in that too the intensity of staining varied in the different fibres and the distribution pattern closely resembled that of succinic dehydrogenase. Whatever be the function of alkaline phosphatase in the muscle, our studies show that the concentration of this enzyme in muscle fibres follow the concentration pattern of the oxidative enzymes and is related to activity. It should therefore be concluded that the broad glycogen-loaded white fibres in the breast muscle of the bat do not resemble those in the pigeon breast muscle as far as their enzymic activity is concerned, and the condition in pigeon is an extreme case of specialisation, the exact significance of which is well worth investigating.

Summary

1. The succinic dehydrogenase and alkaline phosphatase activity in the *pectoralis major* muscle of a Rhinolophid bat (*Hipposideros speoris*) was studied histochemically.

2. It was found that the concentration of these enzymes vary in the different fibres and bear a relationship with the diameter and mitochondrial content of the fibres.

3. The activity of these enzymes and mitochondrial content tend to decrease as the diameter of the fibre increases.

4. The enzyme activity of this muscle is compared with that of the pigeon breast muscle.

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STUDIES ON GASTRIC SECRETORY FUNCTION IN NORMALS

Part I

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RESULTS of gastric secretory function by fractional gastric analysis method with alcohol meal have been previously reported from this laboratory (Pai, 1956 and 1957). Further results obtained in some normal subjects studied are presented here.

Materials and methods

Twenty-eight normal healthy male subjects, mostly from amongst medical students, of age varying between eighteen and twenty-four years, have been studied for their gastric secretory function. A majority of these subjects, were from Gujarat (Bombay State) and were taking mostly vegetarian type of diet, supplemented by milk, though they had no objection to taking eggs, which, however, they took on very rare occasions. Only two subjects belonged to the non-vegetarian group taking a mixed type of diet, with fish and/or meat-preparation included in their meals, on about two days in a week on the average; otherwise their diet was similar to that of the rest.

The method used was the fractional gastric analysis with alcohol meal after Bloomfield and Keefer (1927), which was standardized here in the laboratory and reported earlier (Pai, 1957). The examination included measuring of the volume of the residuum, the quantitative estimation of free and total acidity and the qualitative tests for the presence or otherwise of blood bile, mucus, lactic acid and starch in all the samples aspirated. The results of the above examination are given in the tables, I-III.

Results and discussions

Residuum :

The volume of the fasting juice varied between 5 and 155 cc. with an average of 40.5 cc. This volume has been found to vary within wide limits as reported by various workers. Bennet and Ryle (1921) obtained a range of 10 cc. to 150 cc. with an average of 54 cc. in 39 subjects. Bhatia,

TABLE I

Results of fractional gastric analysis after alcohol meal.

Sr. No.	Subjects	Age in years	Highest acidity in cc N/10 acid %.		Time at which highest acidity reached		Volume of fasting juice in cc
			Free	Total	hour	mts.	
1	M1	20	78.0	80.0	0	45	24.0
2	N.	21	28.0	33.0	2	30	22.0
3	J1	21	50.0	56.0	0	0	74.0
4	V1	18	34.0	42.0	0	45	60.0
5	P1	19	76.0	82.0	2	45	43.0
6	K.	20	18.0	22.0	1	0	47.0
7	R1	23	0.	8.0	0	0	49.0
8	S1	18	24.0	30.0	0	45	14.0
9	R2	20	16.0	24.0	1	15	27.0
10	M2	19	86.0	92.0	2	30	5.0
11	J2	20	30.0	44.0	0	45	30.0
12	R3	19	54.0	60.0	1	15	6.0
13	L.	18	58.0	64.0	0	0	78.0
14	D1	18	52.0	56.0	0	15	53.0
15	R4	19	74.0	80.0	1	0	32.0
16	C1	19	38.0	46.0	0	0	48.0
17	G.	20	62.0	66.0	1	45	52.0
18	J3	22	62.0	70.0	0	0	39.0
19	P2	21	56.0	64.0	0	45	28.0
20	C2	20	36.0	44.0	0	0	155.0
21	V2	18	12.0	20.0	0	45	42.0
22	M3	24	40.0	46.0	2	30	40.0
23	C3	20	30.0	34.0	0	45	16.0
24	M4	19	38.0	50.0	2	15	36.0
25	D2	21	28.0	36.0	0	0	50.0
26	C4	20	20.0	34.0	0	45	38.0
27	S2	19	36.0	42.0	1	15	12.0
28	S3	18	24.0	34.0	0	15	19.0

TABLE II

Results of fractional gastric analysis examination showing the percentage incidence of the presence of various conditions etc.

	Presence of						
	Achlorhydria	Hyperchlorhydria	Blood	Bile	Mucus	Starch	Lactic acid
Percentage incidence of	4 %.	14 %.	7 %.	35 %.	18 %.	7 %.	nil.

Patel and Dundas (1931) observed a range of 2.7 cc. to 110 cc. with an average of 28.5 cc. Manglik, Goel and Manglik (1942) reported that the average volume of fasting contents was 30 cc., in Northern Indian subjects. The efficiency of the emptying power of the stomach is indicated by the volume of the fasting juice. None of the samples of the fasting juice tested showed the presence of lactic acid or evidence of foul odour, which are taken as indicative of fermentation in the stomach.

Subsequent samples :

The highest free acidity in the gastric samples aspirated ranged between 0 to 86 cc. N/10 acid % with an average value of 41.0 cc. N/10 acid %. The highest total acidity in the samples ranged between 8 and 92 cc. N/10 acid % with an average value of 48.0 cc. N/10 acid %. This acid response obtained in the present series is compared with that obtained by other workers as shown in the table below.

TABLE III

Comparison of gastric response obtained by various workers

Series	Type of meal	Gastric acidity in cc. N/10 acid %	
		Maximum (highest) total acidity	Mean (highest free acidity)
Bhatia et al (loc.cit.)	Gruel	58	20,23
Mangalik et al (loc.cit.)	Alcohol	118	40
Napier and Das Gupta (1935)	Gruel	90	42,1
Rao (1937)	Gruel	108	42.0
Present series	Alcohol	92	41.0

The peak of the total acidity was attained averagely at the one hour period after the administration of the meal. Napier, Chaudhari and Rai Chaudhari (1938) classified the response of highest free acidity above 65 cc. N/10 acid % as hyperchlorhydric. Accordingly in the present series 14 % of the subjects gave hyperchlorhydric response. Rao (1937) had obtained 15 % incidenee of hyperchlorhydria and 5 % of achlorhydria, in his subjects. The incidence of achlorhydria in the present series was 4 %. Taking all sample together, starch was found to be present in 7 % mucus in 18 %, bile in 35 %, blood in 7 %, which may be due to trauma during introduction of the stomach tube, whereas none of the samples showed the presnce of lactic acid (*vide* Table II).

Summary and Conclusions

(1) Twenty-eight normal healthy subjects, varying between eighteen and twentyfour years of age, were studied for their gastric secretory function by the fractional gastric analysis method. The usual diet of a Majority of these subjects was a vegetarian type supplemented by milk.

(2) The average highest free acidity was 41.0 cc and the corresponding total acidity 48.0 cc of N/10 acid %. The average volume of fasting juice was 28.5 cc.

(3) The peak of the total acidity was attained on the average at one hour period after the alcohol meal was given. The incidence of alcohohydria was 4 % and that of hyperchlorhydria was 18 % amongst the subjects studied.

(4) The results obtained are compared with those of other workers and the significance of the findings discussed.

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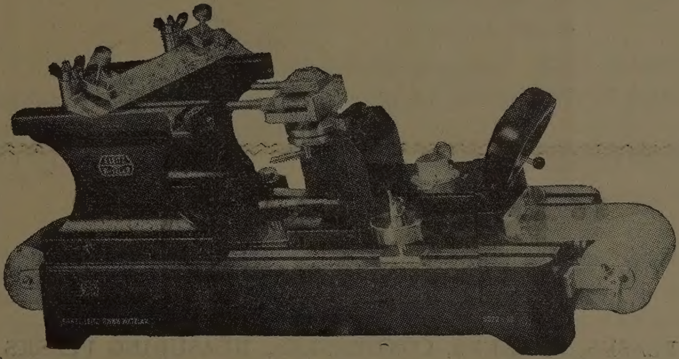

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